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Short communication

Rapid quantitative analysis of oxiracetam in human plasma by liquid chromatography/electrospray tandem mass spectrometry

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Abstract

A rapid and accurate reversed-phase liquid chromatography–tandem mass spectrometry (LC–MS/MS) method has been developed and validated for the quantitative determination of oxiracetam in human plasma. After addition of internal standard (piracetam) plasma was precipitated with two volumes of acetonitrile and the supernatant was evaporated. The residues were dissolved in 0.1% acetic acid and analyzed by reversed-phase HPLC with the detection of the analyte in the multiple reaction monitoring (MRM) mode. This method for the determination of oxiracetam was accurate and reproducible, with a limit of quantitation of 0.2 μg/ml in human plasma. The standard calibration curve for oxiracetam was linear ($r^2 = 0.999$) over the concentration range 0.2–40.0 μg/ml in human plasma. The intra- and inter-day precision over the concentration range of oxiracetam was lower than 8.3% (relative standard deviation, %R.S.D.), and accuracy was between 92.5–106.4%.

Keywords: Oxiracetam; Pharmacokinetics; LC–MS/MS; Multiple reaction monitoring

1. Introduction

Oxiracetam (4-hydroxy-2-oxo-1-pyrrolidine acetamide) is a nootropic agent, which is used for the treatment of various cognitive disorders. This drug is also known to improve both learning and memory processes [1–4]. The analytical tools developed for the determination of oxiracetam in biological matrices include normal-phase liquid chromatography with UV detection [5], reversed-phase liquid chromatography with fluorescence detection followed by derivatization [6], and column-switching high-performance liquid chromatography [7]. These reported methods, however, are not adequate for pharmacokinetic studies due to relatively lower selectivity and sensitivity of short-wavelength UV detection system and tedious sample pretreatment, such as a fluorescent derivatization. Therefore, a simple and sensitive analytical tool for the determination of oxiracetam in human plasma is required to overcome such restrictions.

Recently, tandem mass spectrometry has been widely used for the determination of various compounds because of its inherent accuracy and excellent sensitivity and selectivity [8]. Considering these points, an advanced reversed-phase LC–MS/MS technique combined with rapid sample preparation by simple precipitation seems to be the best candidate for the quantitation of oxiracetam. To our knowledge, no methods have been reported to determine oxiracetam in human plasma by reversed-phase LC–MS/MS with multiple reaction monitoring (MRM).

This paper describes a simple and sensitive LC–MS/MS technique for the quantitation of oxiracetam in human plasma.

2. Materials and methods

2.1. Materials

Oxiracetam reference standard and the internal standard piracetam were purchased from Sigma Chemicals (Fig. 1) (St. Louis, MO, USA). Human plasma was obtained from

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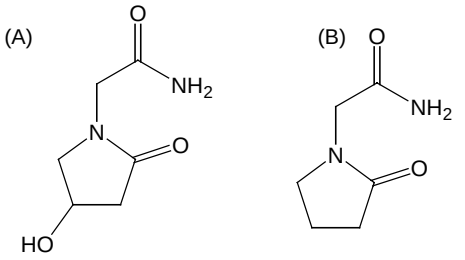

Fig. 1. Structures of (A) oxiracetam and (B) piracetam (IS).

Korea Blood Bank Corp. (Seoul, Korea). Methanol and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were of analytical grade and used as received. HPLC-grade water was prepared using a Milli-Q purification system (Millipore; Bedford, MA, USA). High-purity nitrogen (99.9999%) was purchased from Shin Yang Gas Co. (Seoul, Korea).

2.2. Standard solutions

Standard stock solutions of oxiracetam and piracetam were made up at 1.0 mg/ml in methanol as their free forms. They were further diluted with methanol to obtain working standard solutions at several concentration levels. The calibration curves were obtained, using eight calibration standards, i.e., spiked plasma samples prepared by addition of the stock solution to blank human plasma giving final concentrations of oxiracetam of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, and 40.0 μg/ml.

2.3. Sample extraction

Piracetam (20 μl of 10 μg/ml) was added as an internal standard to 200 μl of plasma sample and 400 μl acetonitrile was added to each human plasma and blank samples. The samples were vortexed for 30 s. and centrifuged for 5 min at 13,000 rpm. The supernatant was taken and evaporated to dryness under nitrogen gas in a TurboVap evaporator (Zymark, Hopkinton, MA, USA). The dried residue was dissolved in 100 μl of 0.1% acetic acid and 4.0 μl was injected into the LC–MS/MS system.

2.4. Reversed-phased liquid chromatography–mass spectrometry

The HPLC was performed using LC-10ADvp binary pump system, SIL-10ADvp autosampler and CTO-10ASvp oven (Shimadzu, Kyoto, Japan). The analytical column was a C_{18} column (Atlantis^{TM} dC_{18}, 50 × 3.0 mm i.d., 3 μm, Waters, Mass, USA). The HPLC mobile phases consisted of 0.1% acetic acid (A) and 100% methanol (B). The flow rate was 0.3 ml/min. Separations were conducted using a gradient: 1% B for 1 min, followed by a linear increase to 80% B over 1 min, then maintained for 1 min. The column was re-equilibrated to initial condition for 1.5 min.

The HPLC was coupled to an API2000 triple-quadrupole mass spectrometry (Applied Biosystems SCIEX, Concorde, Canada) equipped with a Turbo Ion Spray source. Electrospray ionization (ESI) was performed in the positive mode with nitrogen as the nebulizing, turbo spray and curtain gas with the optimum value set of 40, 80, and 40 (arbitrary values). The heated nebulizer temperature was set at 425 °C. The mass spectrometer operated with low and unit resolution for Q1 and Q3, respectively. MRM detection was employed using nitrogen as the collision gas (four arbitrary value) with a dwell time of 150 ms for each transition, monitoring the transition of the protonated molecular ion *m*/*z* 159 and *m*/*z* 143 to their corresponding product ion *m*/*z* 114 and *m*/*z* 126 for oxiracetam and piracetam (IS), respectively. Collision energy was set to be 19 and 13 eV for oxiracetam and piracetam, respectively. The data was acquired, using Analyst 1.3.1 software.

2.5. Calibration and validation

The calibration curves for oxiracetam in human plasma were generated by plotting the peak area versus the concentrations in the standard spiked plasma samples by least-square linear regression (no weighting). The calibration curves consisted of eight calibration standards and each standard was prepared in triplicate. Intra-day coefficient of variation (CV) and accuracy of the method were evaluated by the analysis of five plasma samples spiked with 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, and 40.0 μg/ml. The CV and accuracy for inter-day assay were assessed at the same concentration, and repeated for five different days.

2.6. Human pharmacokinetic study

Twenty-four healthy male volunteers, whose age was between 20 and 28 were selected for this study. Informed consent forms were signed according to institutional guidelines. This study was approved by the Human Subjects Committee of Kyunghee University, and was conducted at the Kyunghee Medical Center. Oxiracetam tablet (800 mg) was orally administered to all volunteers with 200 ml water and blood was collected at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h postdose. Plasma was harvested after centrifugation and stored frozen at −20 °C until analyzed. The pharmacokinetic parameters were determined using standard non-compartmental methods. Plasma AUC was calculated, using WinNonlin (version 3.1, Scientific Consulting, KY, USA) with trapezoidal method.

3. Results and discussion

3.1. Sample preparation and analysis

Rapid sample preparation and analysis is especially important for the pharmacokinetic studies that require a large

number of sample analyses. Oxiracetam is very polar compound, of low molecular mass, and is not easy to chromatograph whilst the addition of pH modifiers often results in good retention of polar compounds on reversed-phase columns. Oxiracetam was not readily retained and showed a poor chromatographic properties. However, the use of an Atlantis dC_{18} column, developed for a convenient analysis of polar compounds, provided the required retention. Under our chromatographic condition, oxiracetam had a retention time at 1.3 min and generated an excellent peak shape for quantitative analysis.

Recovery of oxiracetam was relatively low either in solvent extraction or solid-phase extraction. In addition, it was difficult to obtain sufficient separation of the oxiracetam from interfering compounds in plasma. The high sensitivity and selectivity of MS/MS allowed a simple solvent precipitation, to be used providing high recoveries (data not shown).

The product ion mass spectra and the postulated fragmentation patterns of oxiracetam and piracetam are illustrated in Fig. 2. Loss of NH_3 (17 amu) from the protonated oxiracetam (*m*/*z* 159) and piracetam (*m*/*z* 143) yielded fragment ions at *m*/*z* 142 and 126, respectively. The loss of CO (28 amu) from the fragment ion at *m*/*z* 142 and 126 resulted in fragment ions at *m*/*z* 114 and 98, respectively. Among these product ions, the most abundant ions (*m*/*z* 114 for oxiracetam and *m*/*z* 126 for piracetam) were selected for MRM analysis.

The specificity and selectivity of the method were investigated by preparing and analyzing human plasma blanks from four different batches of pooled human plasma. No interference was observed in drug-free plasma samples (Fig. 3A) at the retention times of oxiracetam and piracetam. In addition carry-over was not observed in our system. A significant carry-over effect was noted when acetonitrile was used as organic modifier. Fig. 3B shows the MRM chromatograms obtained by the analysis of the plasma spiked with 10 μg/ml oxiracetam and 1.0 μg/ml internal standard.

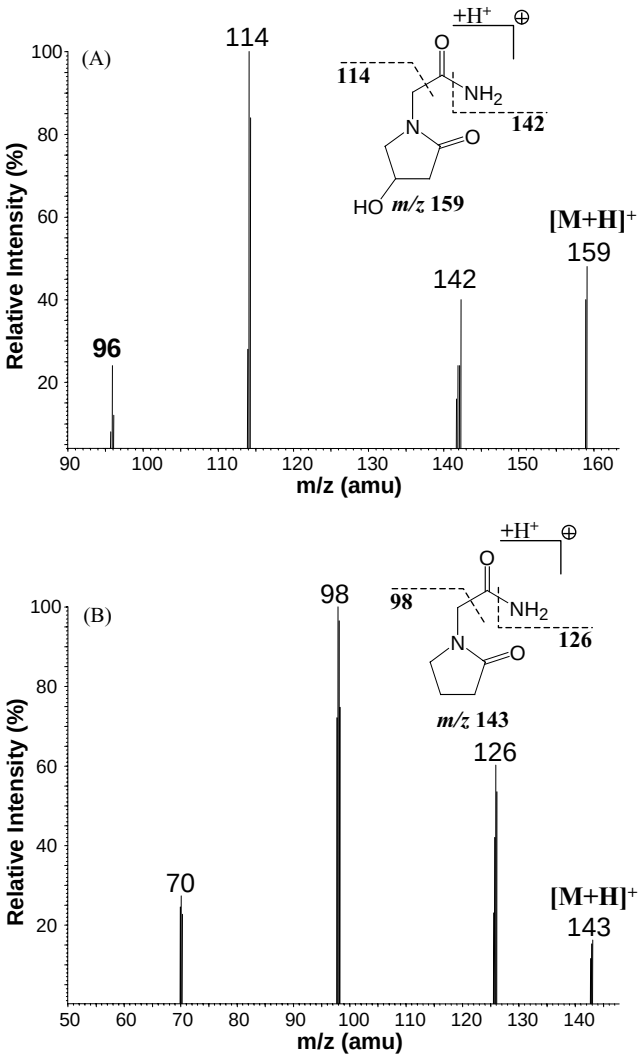

Fig. 2. Product ion mass spectra of $[M+H]^+$ ions of (A) oxiracetam and (B) piracetam (IS).

3.2. Method validation

The calibration curve was constructed using ten different concentrations and processed by least-square linear regression analysis (no weighting). The standard calibration curve for spiked human plasma containing oxiracetam was linear over the concentration range 0.2–40 μg/ml with a correlation coefficient greater than 0.999.

Table 1
Intra-day and inter-day coefficient of variation and accuracy for determination of oxiracetam in human plasma (N = 5)

Theoretical concentration (μg/ml)	Intra-day			Inter-day		
	Concentration found (μg/ml) (mean ± S.D.)	CV (%)	Accuracy (%)	Concentration found (μg/ml) (mean ± S.D.)	CV (%)	Accuracy(%)
0.2	0.19 ± 0.02	8.3	92.5	0.20 ± 0.01	3.8	98.2
0.5	0.50 ± 0.03	5.3	99.9	0.47 ± 0.02	4.4	93.6
1.0	1.06 ± 0.06	6.1	106.3	0.99 ± 0.06	5.6	99.0
2.0	2.13 ± 0.08	3.8	106.4	2.01 ± 0.11	5.5	100.3
5.0	5.07 ± 0.11	2.2	101.3	4.82 ± 0.13	2.8	96.3
10.0	11.1 ± 0.3	2.3	110.5	10.4 ± 0.4	3.6	103.8
20.0	20.7 ± 0.6	2.9	103.7	19.4 ± 0.6	3.3	96.9
40.0	39.7 ± 1.0	2.5	99.4	38.1 ± 1.6	4.2	95.4

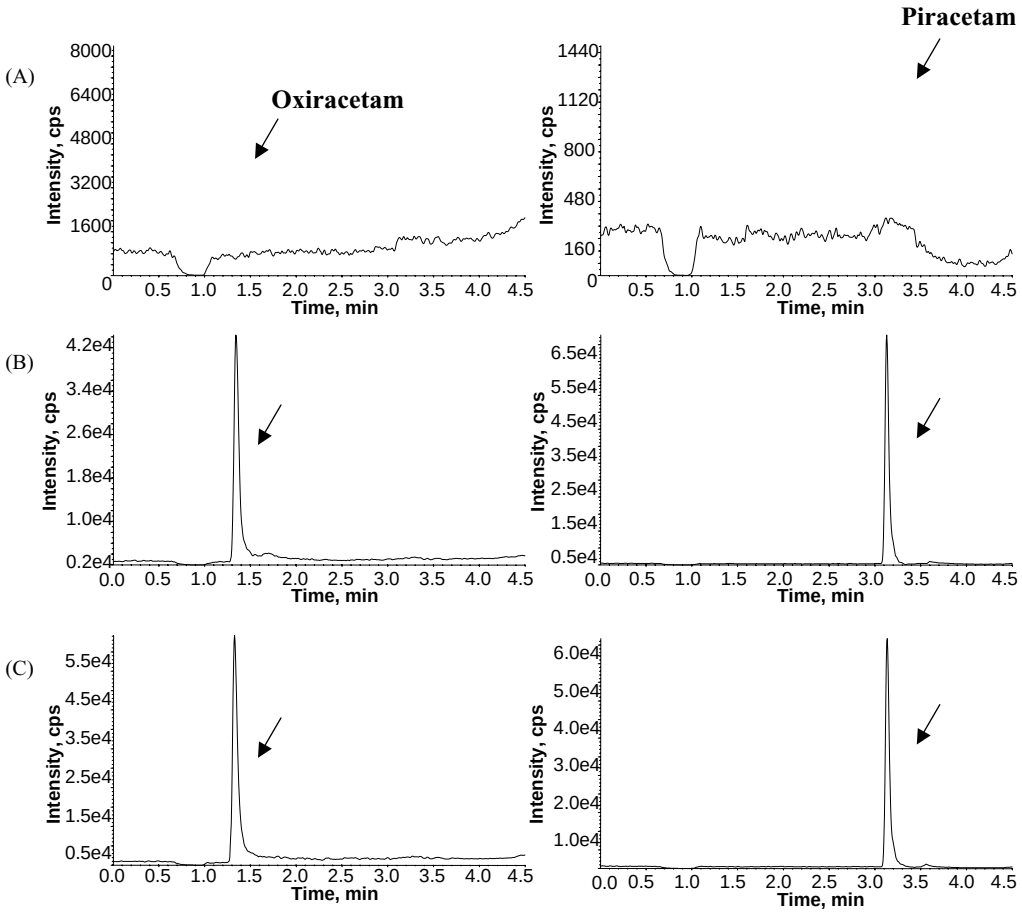

Fig. 3. LC–MS/MS chromatograms of (A) blank human plasma, (B) plasma spiked with oxiracetam (10 μg/ml) and IS (1.0 μg/ml), and (C) plasma collected 2.0 h after single oral administration of oxiracetam tablet (800 mg).

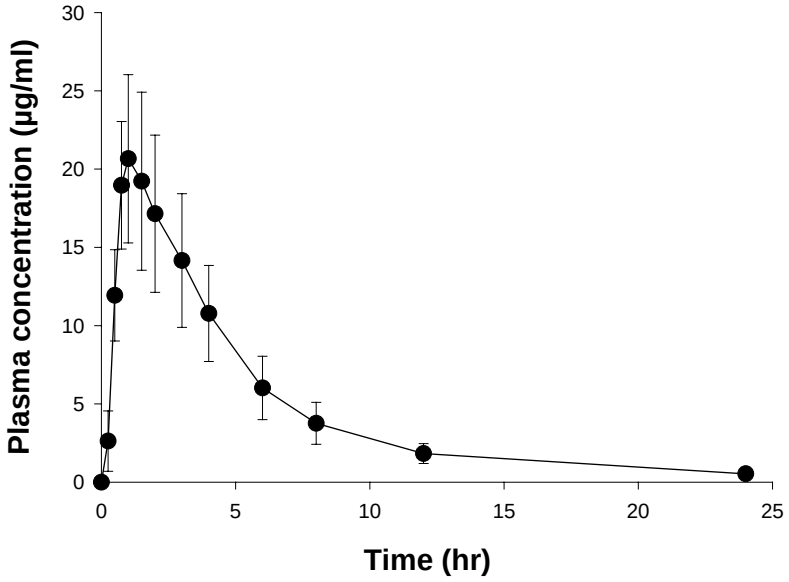

Fig. 4. Mean plasma concentrations of oxiracetam after oral administration of oxiracetam tablet (800 mg) to 24 healthy volunteers. Each point represents the mean ± S.D.

The intra- and inter-day variations of oxiracetam determination in human plasma are summarized in Table 1. The intra-day coefficients of variation were between 2.2 and 8.3% and the accuracies ranged from 92.5 to 106.4%. The inter-day coefficients of variation were between 2.8 and 5.6% and the accuracies were between 93.6 and 103.8%. Freeze/thaw stability and 24-h temperature stability were also evaluated and the differences were within 3%. Under these conditions, a lower limit of quantitation (LLOQ) of 0.2 μg/ml was achieved for oxiracetam, using a 0.2-ml plasma sample volume. This was the lowest concentration of the analyte that can be measured with a coefficient of variation and accuracy both less than 15%. This LLOQ was sufficient for pharmacokinetic studies.

3.3. Pharmacokinetic investigation in healthy volunteers

The validated method was applied to determine the concentration of oxiracetam in human plasma after single oral administration of 800 mg oxiracetam in tablet to 24 healthy volunteers. Mean plasma concentration–time curve of oxiracetam after oral administration of oxiracetam is shown in Fig. 4. The AUC of oxiracetam was 110 ± 28 μg h/ml and the average C_{max} of oxiracetam was 21.6 μg/ml.

4. Conclusions

The LC–MS/MS method combined with protein precipitation has been developed for the quantitative determination of oxiracetam in human plasma. The present method affords the sensitivity, accuracy and precision necessary for quantitative measurements in pharmacokinetic studies and therapeutic monitoring of oxiracetam.

Acknowledgements

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